

Supplemental information

***Streptococcus pneumoniae* pneumolysin
and neuraminidase A convert high-density
lipoproteins into pro-atherogenic particles**

Shahan Syed, Eija Nissilä, Hanna Ruhanen, Satoshi Fudo, Meztlli O. Gaytán, Sanna P. Sihvo, Martina B. Lorey, Jari Metso, Katariina Öörni, Samantha J. King, Oommen P. Oommen, Matti Jauhiainen, Seppo Meri, Reijo Käkelä, and Karita Haapasalo

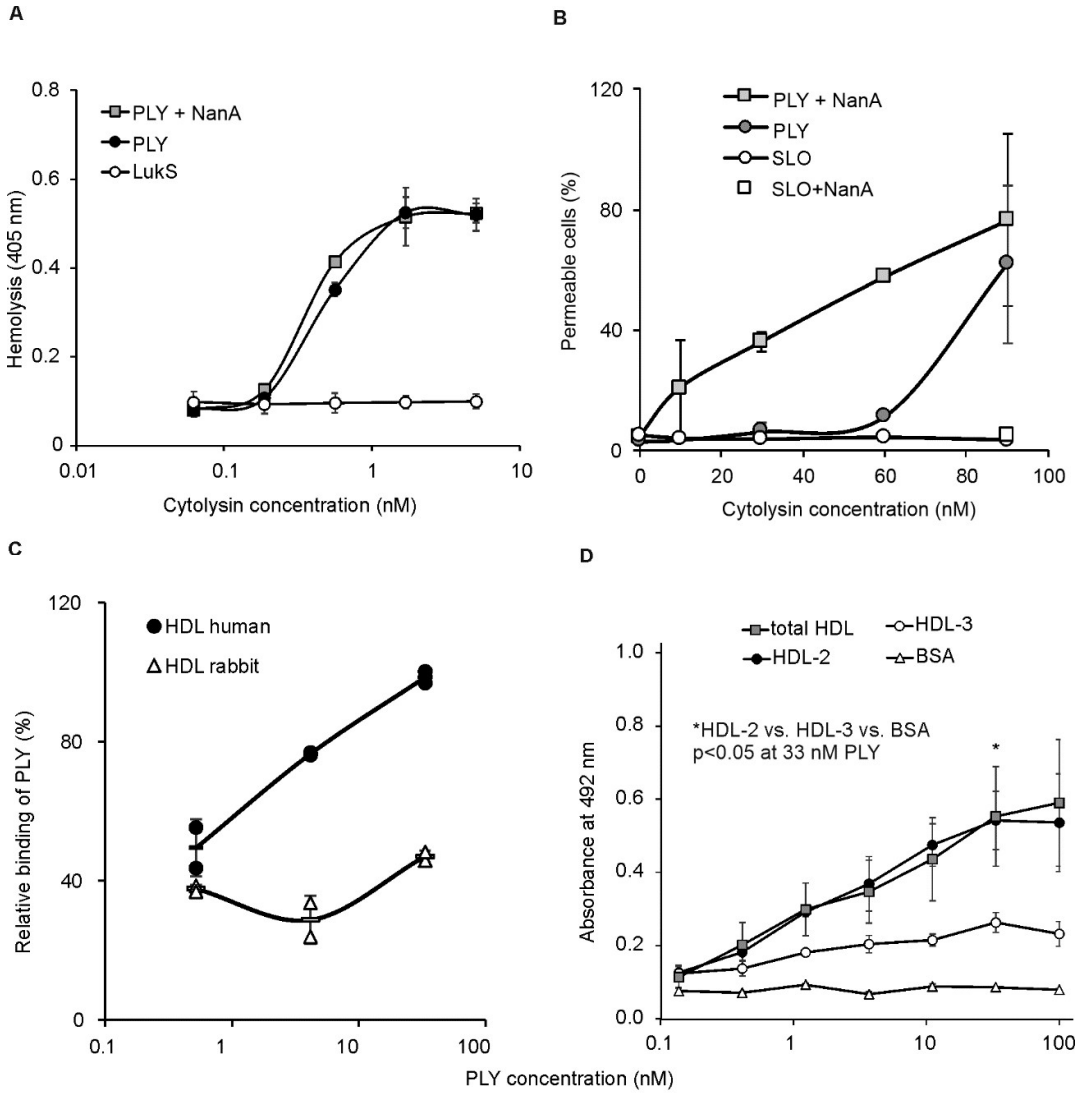


Figure S1. Lysis of red blood cells and cultured monocytic cells by PLY and NanA and binding of PLY to different HDL particles (Related to Figure 1). **A**, Red blood cells isolated from hirudin anticoagulated blood were incubated with increasing concentrations of (x-axis) cytolysins PLY or LukS negative control in the presence or absence of NanA (800 nM). Hemolysis is shown as increase in absorbance at 405 nm. **B**, Cultured monocytic U937 cells were incubated with increasing concentrations of PLY with or without NanA (800 nM). Here, *C. perfringens* streptolysin O (SLO) that cannot lyse cells without a reducing agent was used as a negative control. Permeability is shown as percentage of cells stained positive for DAPI in flow cytometry. Error bars indicate SD calculated from at least two biological replicates. Binding of PLY to **(C)** rabbit HDL, **(C and D)** human total HDL, **(D)** HDL-2, HDL-3 and BSA coated microtiter plates was analyzed by ELISA as described in materials and methods. **(C)** Binding % is calculated as relative to 100% binding to HDL-2 at 33 nM PLY concentration. **(D)** The detected statistical significant differences ($p < 0.05$) at 33 nM PLY concentration was calculated using one-way ANOVA followed by Dunnett's non-parametric multiple comparison test. Error bars indicate SD values calculated from three repeated experiments.

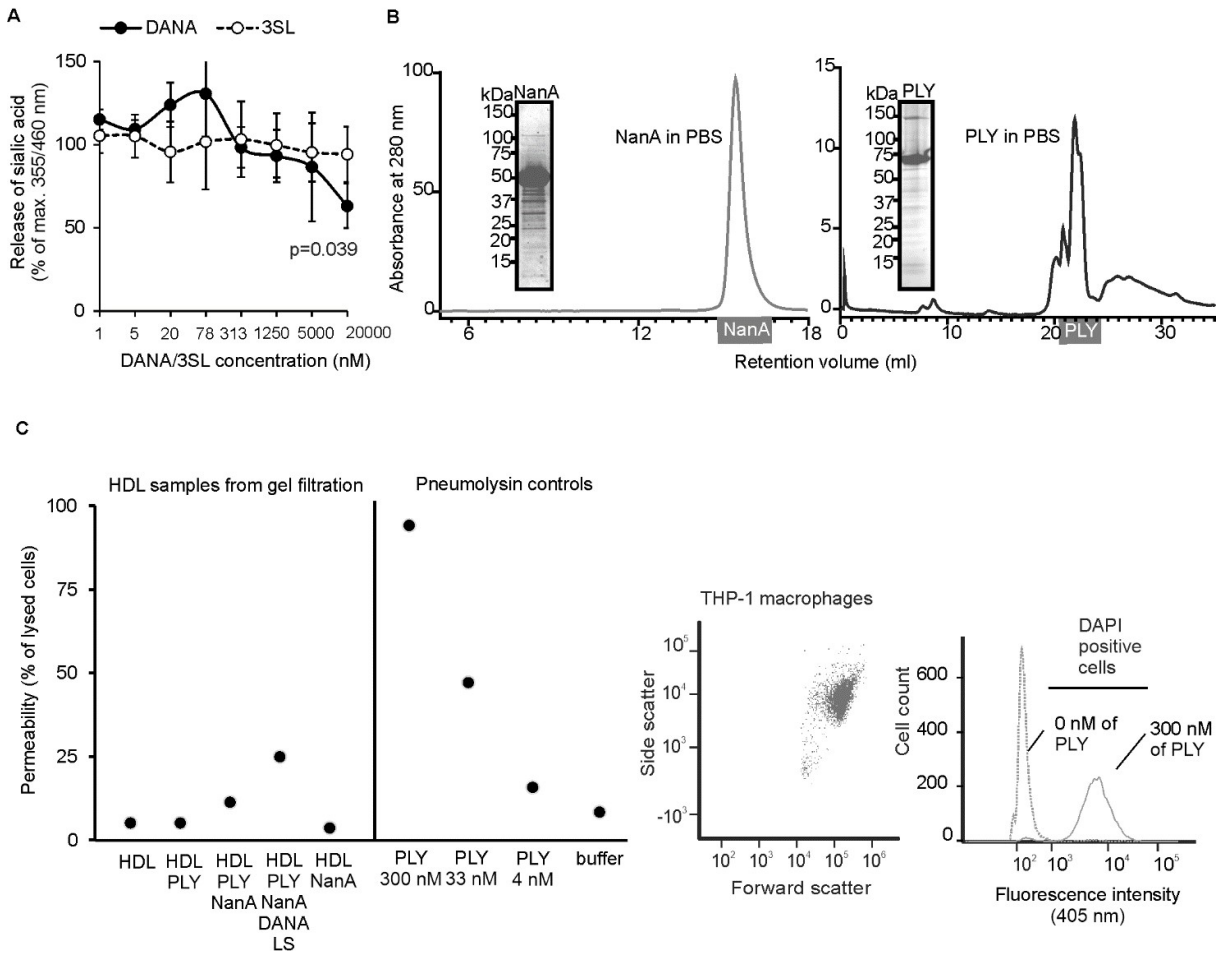


Figure S2. Activity of NanA inhibited by DANA and lytic activity of PLY in the HDL-2 fractions after gel filtration (Related to Figure 3). **A**, Inhibition of NanA using increasing concentrations of neuraminidase inhibitor 2,3-didehydro-2-deoxy-N-acetylneuraminic acid (DANA). Sialic acid analog 3'-Sialyllactose (3SL) showed no inhibitory activity towards NanA. Release of sialic acids is calculated as % of maximum absorbance measured at the lowest DANA/3SL concentration. The detected statistical significance ($p < 0.05$) between highest and lowest NanA concentrations were calculated using Mann-Whitney U-test. Error bars showing SDs are calculated from at least three biological replicates. **B**, PLY and NanA were run in the same conditions as the HDL-2 samples in the study. NanA eluted at retention volumes between 14.5-15 ml, while PLY eluted at retention volumes between 20-23 ml. PLY and NanA run in SDS-PAGE with the kDa are shown with molecular weight marker. **C**, Cultured monocytic THP-1 cells were incubated with different concentrations of PLY and with 5 μ g of the HDL-2 samples incubated with or without PLY, NanA, DANA and/or Liposomes (LS). Permeability is shown as percentage of lysed cells stained positive for DAPI in flow cytometry. Some cytolytic activity can be seen in the sample with PLY and NanA inhibitors when compared to the buffer background. (upper right) Density plot of THP-1 cells and (lower right) histogram of background cells (0 nM of PLY) and DAPI positive cells (300 nM of PLY) used for calculation of permeability % in FlowJo.

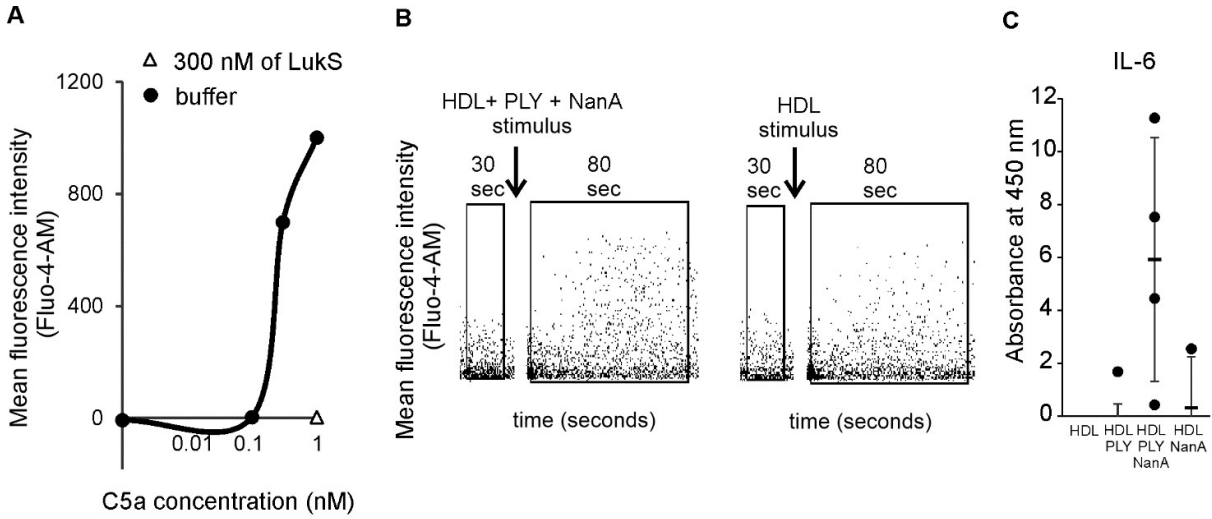


Figure S3. Calcium mobilization of HEK-C5aR cells (Related to Figure 4). **A**, Calcium mobilization induced by active C5a at 0-1 nM concentrations in the presence or absence of 300 nM LukS. **B**, Gating of Fluo-4-AM labeled HEK-C5aR cells before and after addition of the stimulus (HDL-2 particles incubated with or without PLY and NanA). **C**, Supernatants of HUVEC cell cultures incubated with HDL, HDL+PLY, HDL+PLY+NanA and HDL+NanA subjected to ELISA assay. IL-6 was released in the presence of HDL+PLY+NanA. The assay was repeated four times.

Table S1. Protein identification of plasma isolated total HDL separated from blood incubated with or without pneumolysin or pneumolysin and neuraminidase A (Related to Figure 1E).

Incubated with pneumolysin and neuraminidase A						
Accession	Description	*Score	Coverage	# Unique Peptides	# PSMs	MW [kDa]
P02647	Apolipoprotein A-I	261.39	76.40	30	138	30.8
P02671	Fibrinogen alpha chain	81.43	25.98	18	47	94.9
P69905	Hemoglobin subunit alpha	81.90	90.85	11	34	15.2
P68871	Hemoglobin subunit beta	75.17	81.63	8	32	16.0
P02649	Apolipoprotein E	28.20	36.91	13	22	36.1
P02652	Apolipoprotein A-II	46.58	72.00	9	21	11.2
P02654	Apolipoprotein C-I	19.62	42.17	7	20	9.3
P0DJ18	Serum amyloid A-1	46.87	56.56	5	20	13.5
P01024	Complement C3b	35.74	4.87	7	17	187.0
P02042	Hemoglobin subunit delta	31.35	69.39	4	16	16.0
O14791	Apolipoprotein L1	15.31	13.07	5	14	43.9
P35542	Serum amyloid A-4 protein	21.87	40.00	5	11	14.7
P02655	Apolipoprotein C-II	14.78	52.48	6	10	11.3
P06727	Apolipoprotein A-IV	9.69	25.76	10	10	45.4
P02656	Apolipoprotein C-III	30.95	37.37	4	9	10.8
P10909	Clusterin	12.27	16.26	8	9	52.5
P01009	Alpha-1-antitrypsin	1.77	14.59	6	9	46.7
P0C0L4	Complement C4-A	12.39	4.64	5	9	192.7
P27169	Serum paraoxonase/arylesterase 1	11.68	24.23	8	9	39.7
P02675	Fibrinogen beta chain	20.45	4.07	2	8	55.9
P02766	Transthyretin	11.27	49.66	6	6	15.9
O95445	Apolipoprotein M	5.88	22.87	5	5	21.2
Q14624	Inter-alpha-trypsin inhibitor heavy chain H4	2.96	5.16	3	4	103.3
P02775	Platelet basic protein	12.28	11.72	2	4	13.9
P02768	Albumin	0.00	5.58	3	4	69.3
Pneumolysin**	sequence	8.47	13.40	4	4	52.7
P04114	Apolipoprotein B-100	2.04	0.68	2	2	515.3
P04180	Phosphatidylcholine-sterol acyltransferase	3.50	9.09	2	2	49.5
P00739	Haptoglobin-related protein	3.07	5.17	1	1	39.0
Q9BUN1	Protein MENT	2.99	4.40	1	1	36.7
Incubated with buffer						
Accession	Description	*Score	Coverage	# Unique Peptides	# PSMs	MW [kDa]
P02647	Apolipoprotein A-I	308,22	78,65	37	164	30,76
P02671	Fibrinogen alpha chain	56,47	18,24	14	33	94,91
P02652	Apolipoprotein A-II	55,14	72	9	26	11,17
P02649	Apolipoprotein E	39,56	34,38	14	24	36,13
P02654	Apolipoprotein C-I	18,13	42,17	7	18	9,33
P35542	Serum amyloid A-4 protein	23,56	53,85	8	16	14,74
P02656	Apolipoprotein C-III	36,89	58,59	7	14	10,85
P06727	Apolipoprotein A-IV	11,10	32,58	13	13	45,37
P02655	Apolipoprotein C-II	17,18	56,44	7	12	11,28
P0C0L5	Complement C4-B	15,57	8,94	1	12	192,63

P0C0L4	Complement C4-A	13,55	9,06	1	12	192,66
P0DJ18	Serum amyloid A-1 protein	44,57	56,56	3	12	13,52
P27169	Serum paraoxonase 1	11,48	28,17	8	11	39,71
O14791	Apolipoprotein L1	13,82	15,08	6	9	43,95
P69905	Hemoglobin subunit alpha	18,55	30,28	5	9	15,25
P04114	Apolipoprotein B-100	11,16	1,97	8	8	515,28
P10909	Clusterin	9,49	16,26	7	7	52,46
P0DJ19	Serum amyloid A-2 protein	16,75	36,07	1	7	13,52
P68871	Hemoglobin subunit beta	9,81	36,05	5	7	15,99
P02675	Fibrinogen beta chain	13,28	4,07	2	6	55,89
P01024	Complement C3	6,06	3,25	4	6	187,03
P00739	Haptoglobin-related protein	2,22	19,25	5	6	39
P02768	Serum albumin	1,72	6,90	4	6	69,32
P55056	Apolipoprotein C-IV	6,97	28,35	4	4	14,54
P01009	Alpha-1-antitrypsin	6,28	9,33	3	4	46,71
P02766	Transthyretin	4,27	24,49	3	3	15,88
O95445	Apolipoprotein M	2,82	11,17	3	3	21,24
Q13790	Apolipoprotein F	5,45	4,29	1	2	35,38
Q9BUN1	Protein MENT	3,50	6,74	2	2	36,75
P80108	Phosphatidylinositol-glycan-specific phospholipase D	1,84	3,33	2	2	92,28
P02775	Platelet basic protein	3,12	17,19	2	2	13,89
A8MWD9	Putative small nuclear ribonucleoprotein G-like protein 15	0	26,32	1	1	8,54
P0CG39	POTE ankyrin domain family member J	0	0,96	1	1	117,31
Q15465	Sonic hedgehog protein	0	3,46	1	1	49,58
Q8WZA9	Immunity-related GTPase family Q protein	0	3,37	1	1	62,68
P13747	HLA class I histocompatibility antigen, alpha chain E	1,95	3,91	1	1	40,03
Q8TF61	F-box only protein 41	1,80	1,03	1	1	94,44
P04439	HLA class I histocompatibility antigen, A alpha chain	1,98	3,84	1	1	40,82
O95810	Caveolae-associated protein 2	1,97	3,29	1	1	47,14
P01034	Cystatin-C	1,95	7,53	1	1	15,79
Incubated with pneumolysin						
Accession	Description	*Score	Coverage	Unique Peptides	# PSMs	MW [kDa]
P02647	Apolipoprotein A-I	254,62	78,65	31	136	30,76
P02671	Fibrinogen alpha chain	82,83	20,44	19	50	94,91
P68871	Hemoglobin subunit beta	56,68	81,63	6	23	15,99
P69905	Hemoglobin subunit alpha	47,70	70,42	9	21	15,25
P02649	Apolipoprotein E	31,45	33,44	13	20	36,13
P02654	Apolipoprotein C-I	21,87	42,17	7	16	9,33
P0DJ18	Serum amyloid A-1	39,72	59,02	4	16	13,52

P06727	Apolipoprotein A-IV	18,46	32,58	13	15	45,37
P02652	Apolipoprotein A-II	27,01	69	7	15	11,17
P0C0L4	Complement C4-A	19,32	5,96	7	13	192,66
P02656	Apolipoprotein C-III	23,53	39,39	5	9	10,85
P01024	Complement C3	17,31	4,81	5	9	187,03
P35542	Serum amyloid A-4 protein	16,57	40	5	9	14,74
P02042	Hemoglobin subunit delta	13,64	42,18	1	9	16,05
O14791	Apolipoprotein L1	12,64	10,80	4	8	43,95
P0DJ19	Serum amyloid A-2	16,54	36,07	1	8	13,52
P02675	Fibrinogen beta chain	19	4,07	2	7	55,89
P10909	Clusterin	7,87	9,80	6	7	52,46
P27169	Serum paraoxonase 1	9,39	24,23	7	7	39,71
P02766	Transthyretin	7,52	41,50	5	6	15,88
P02655	Apolipoprotein C-II	1,79	28,71	3	5	11,28
O95445	Apolipoprotein M	4,72	22,34	3	4	21,24
P02100	Hemoglobin subunit epsilon	5,63	21,77	1	4	16,19
P02768	Serum albumin	1,77	3,94	2	4	69,32
P02775	Platelet basic protein	8,93	11,72	2	3	13,89
Pneumolysin**	sequence	9,28	10,21	3	3	52,74
Q13790	Apolipoprotein F	1,85	4,29	1	2	35,38
P01009	Alpha-1-antitrypsin	3,62	4,78	2	2	46,71
Q15465	Sonic hedgehog protein	2,04	5,41	2	2	49,58
P49913	Cathelicidin antimicrobial peptide	1,74	5,88	1	1	19,29
P55056	Apolipoprotein C-IV	0	8,66	1	1	14,54
O60503	Adenylate cyclase type 9	2,91	2	1	1	150,60
P00739	Haptoglobin-related protein	1,70	3,74	1	1	39
Q15149	Plectin	0	0,19	1	1	531,47
Q9BUN1	Protein MENT	2,93	4,40	1	1	36,75
P80108	Phosphatidylinositol-glycan-specific phospholipase D	2,45	1,55	1	1	92,28
P61769	Beta-2-microglobulin	2,11	10,92	1	1	13,71
P01034	Cystatin-C	1,69	7,53	1	1	15,79

* Indicates relevance of the protein. Calculated by Proteome Discoverer software according to the score thresholds

Numbers shown as peptide spectrum matches (PSMs) presenting the total number of identified peptide spectra matched for the protein.

**Recombinant *S. pneumoniae* pneumolysin